Journal of Chromatography, 588 (1991) 15–24
Elsevier Science Publishers B.V., Amsterdam

CHROM. 23 659

Assessment of a thermospray interface for the coupling of high-performance liquid chromatography and Fourier transform infrared spectrometry

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(First received April 17th, 1991; revised manuscript received July 30th, 1991)

ABSTRACT

A thermospray interface has been developed to couple high-performance liquid chromatography (HPLC) with Fourier transform infrared (FT-IR) spectrometry. The system has been constructed for operation in normal- and reversed-phase HPLC. The HPLC effluent is thermally desolvated and deposited on to a moving metal substrate, which continually transfers the solutes into the diffuse reflectance (DRIFT) accessory of an FT-IR spectrometer, enabling identification by measurement of the IR reflectance spectrum. Various aspects of the interface design have been investigated and optimised, and the interface has been applied to the analysis of several types of compound, with molecular identification being indicated for the antioxidant Irganox 565 at concentrations lower than 50 $\mu g\ ml^{-1}$.

INTRODUCTION

Over the past decade, the use of combined techniques for solving complex analytical problems has become increasingly popular. This is particularly true in the interfacing of chromatography and molecular spectroscopy. Application of techniques such as gas chromatography–mass spectrometry (GC–MS) is relatively commonplace. Various procedures have been proposed to couple high-performance liquid chromatography (HPLC) with Fourier transform infrared (FT-IR) spectrometry, however this combination has proved much more difficult to achieve than other combined techniques.

Much research work has centred on the use of flow cells [1–3], which can be used in conjunction with some organic solvents. However most solvents commonly used in HPLC possess intense IR absorption bands in important wavelength regions, rendering some spectral regions opaque. In order to maintain a sufficiently high IR transmittance over these regions, the pathlength of the flow cell must be kept short. Hence the sensitivity and amount of qualitative information obtained from a flow cell interface for HPLC–FT-IR may be limited. More favourable results can be obtained if solvent elimination is applied prior to FT-IR detection [4–13].

One of the first solvent elimination techniques de-

0021-9673/91/$03.50

veloped for use in normal-phase HPLC was described by Kuehl and Griffiths [4]. Concentrated portions of the HPLC effluent were deposited into a series of diffuse reflectance cups filled with KBr powder, held in a carousel arrangement. After solvent evaporation, the cup was brought into the IR beam path of the FT-IR spectrometer, and the diffuse reflectance (DRIFT) FT-IR spectrum recorded. Although this system showed good sensitivity, it did not allow continuous analysis of the chromatographic effluent, and a component could be missed or more than one component collected in a single cup. More recently, Wood [5] demonstrated the feasibility of using a particle-beam HPLC–FT-IR interface with normal-phase HPLC solvents, depositing the effluent on to a KBr plate substrate. The most serious limitation of these solvent elimination techniques is that they cannot be used with aqueous based solvents commonly used in reversed-phase HPLC, as water must be removed for any method using KBr as a deposition medium; thus limiting separations to normal-phase or some size-exclusion chromatography.

Various suggestions have been made to circumvent the problems associated with aqueous solvents. Kalasinsky *et al.* [6] removed water from the HPLC column effluent by the continuous addition of 2,2-dimethoxypropane to produce acetone and methanol, which were easily evaporated after deposition on to KCl powder. However, 2,2-dimethoxypropane is expensive and fairly high flow-rates are required. A method of replacing the conventional metal halide substrate with a stainless steel wire mesh was described by Fujimoto *et al.* [7]. Solutes were trapped in the metal net as the solvent was evaporated using a heated gas flow. Unfortunately, the interface was used with an extremely low mobile phase flow-rate of 0.4 μl min^{-1}.

Gagel and Bieman [8,9] have reported a method for narrowbore HPLC–FT-IR where the HPLC effluent is continuously deposited on to and evaporated from the surface of a rotating reflective disc using a nitrogen gas nebuliser. After deposition, the solutes are analysed by rotating the disc in the sample compartment of an FT-IR spectrometer, while the reflectance-absorbance (R-A) spectra are collected. The system displays good sensitivity (110 ng for caffeine) and can deal with gradient elution for up to a 50% aqueous mobile phase. Somson *et al.* [10] proposed a spray jet assembly interface for narro bore HPLC–FT-IR spectrometry using heated trogen gas to promote evaporation of the solve After deposition on to a linearly moving zinc se nide substrate, the deposited compounds were ar lysed by FT-IR microscopy. The system could used with up to 20% aqueous effluents in methar and exhibited high sensitivity (20 ng) for phena threnequinone). However, the interface was us with a relatively low mobile phase flow-rate of 20 min^{-1}. A monodisperse aerosol generation int face (MAGIC) combining LC with FT-IR sp trometry has been described by Robertson, De H seth and Browner [11]. The interface can be us with 100% aqueous solvents and flow-rates of up 0.3 ml min^{-1} with no effluent heating. The sensit ity of this system is around 100 ng for methyl r however, the deposition efficiency is low (abc 10%). Buffers can also be used with this system, b spectral subtraction remains necessary. Details an HPLC–FT-IR interface consisting of a therm spray, moving belt and an optical reflectance acc sory have been reported by Jansen [12]. Applic tions included analysis of various polymers a polymer additives and detection limits in the 100 range were claimed. The system can be used wi 30% water in methanol at a flow-rate of 0.5 min^{-1}, but no information was provided on th mospray deposition efficiency or solute spot si and spreading effects.

Recently, we reported preliminary details of thermospray interface for coupling HPLC with F IR spectrometry using diffuse reflectance optics a a moving ribbon substrate [13], which can be us in both normal- and reversed-phase HPLC. T column effluent is vaporised using a thermospr similar to that used by Vestal to couple HPLC mass spectrometry [14,15], and deposited on to moving metal substrate similar in concept to t transportation device used by Yang *et al.* [1 which continually transfers the de-solvated solut into the diffuse reflectance accessory of the FT-I spectrometer. The interface has been used wi 100% aqueous effluents at flow-rates of 0.5 min^{-1}. Various aspects of the interface design ha been investigated further and the system has be evaluated to illustrate the qualitative and quantit tive capabilities of HPLC–FT-IR in the analysis several types of compounds.

EXPERIMENTAL

Schematic diagrams of the HPLC–FT-IR interface assembly and thermospray are shown in Figs. 1 and 2.

Apparatus

The HPLC system was a Philips PU 4100 liquid chromatograph (Cambridge, UK) utilising a Rheodyne 7125 syringe loading injector (Berkeley, CA, USA) with a 20- or 50-μl sample loop. The analytical columns were either a Hichrom stainless-steel (250 mm × 4.6 mm I.D.) Spherisorb S50DS2 column or a stainless-steel (250 mm × 4.6 mm I.D.) Spherisorb S5NH column (Berkshire, UK). Various compositions of methanol–acetonitrile–water mobile phases were pumped through the columns at flow-rates of 1 or 0.5 ml min^{-1}. A Philips PU 4110 UV–visible detector or Philips PU 4026 differential refractive index detector was used during HPLC method development prior to FT-IR spectrometric detection. A smooth stainless-steel ribbon (width 13 mm, thickness 0.02 mm), which moved with a constant speed of either 1 or 1.5 cm min^{-1}, was used as a substrate for thermospray deposition and transport of solutes.

IR spectrometric data were obtained using either a Philips PU 9800 FT-IR spectrometer or Nicolet system 800 FT-IR spectrometer (Madison, WI, USA), both equipped with a Spectra-Tech Collector diffuse reflectance accessory (Stamford, CT, USA). The Philips instrument was equipped with a deuterated triglycine sulphate (DTGS) detector, and the Nicolet instrument with a medium range mercury-cadmium-telluride (MCT) detector. IR data, in the form of single-scan interferograms, were collected at 8 cm^{-1} resolution and stored using standard Philips (now Unicam) FT-IR computer software. Interferograms were transformed to IR transmittance spectra using the fast Fourier transform algorithm, post-run. Background spectra were collected from the deposition trace on the substrate surface prior to the area of interest. Specially developed Philips (now Unicam) computer software was used to process this data and construct FT-IR functional group chromatograms (FGCs). These were obtained by calculating the integrated IR transmittance across various wavenumber windows (corresponding to particular functional groups), as a function of time.

Effluent from the HPLC was transferred to the thermospray through stainless-steel tubing (250 μm

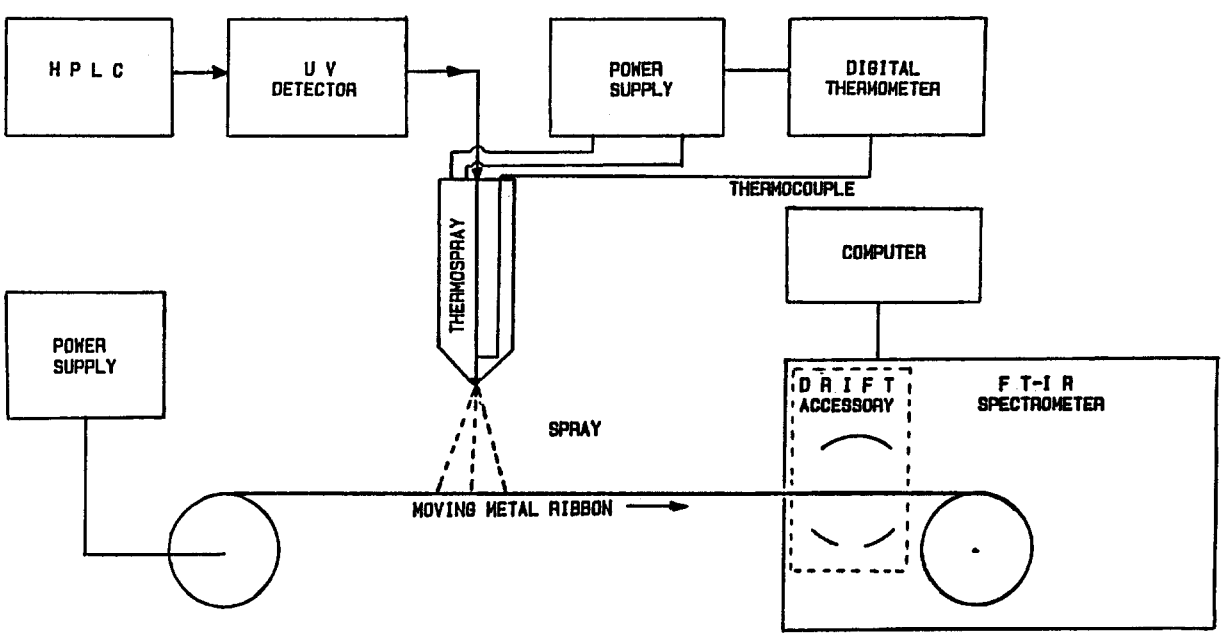

Fig. 1. Schematic diagram of HPLC–FT-IR interface assembly.

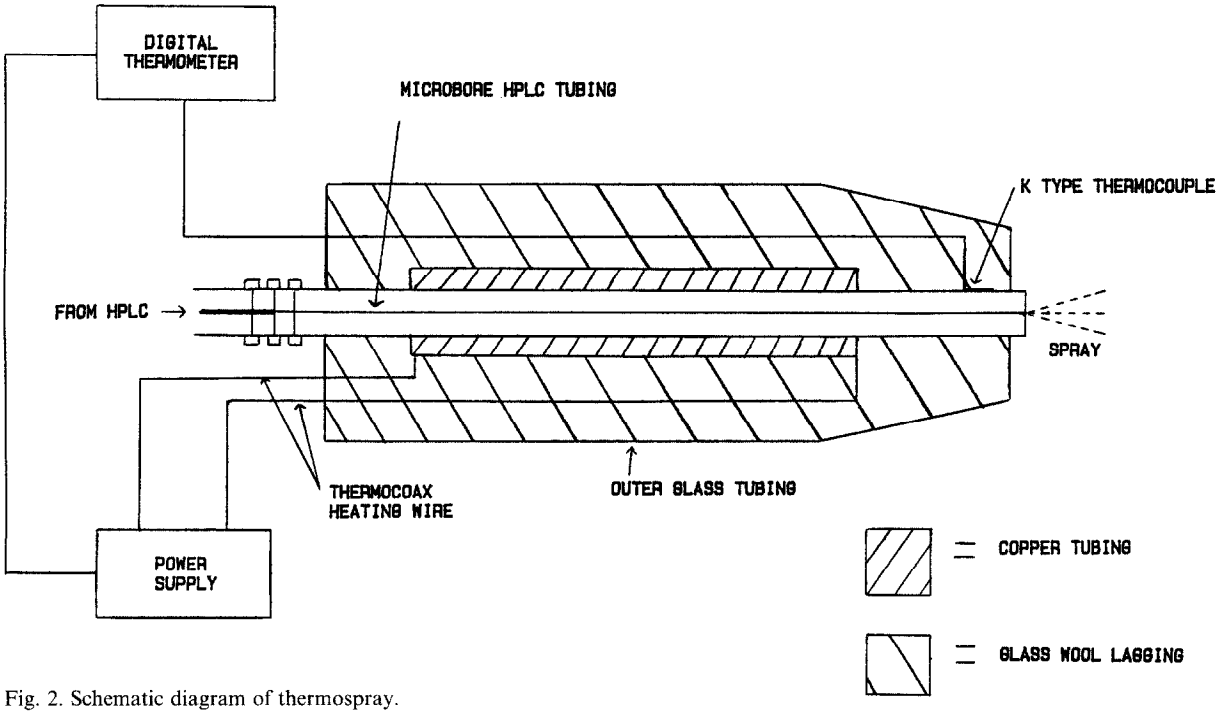

Fig. 2. Schematic diagram of thermospray.

I.D., 1.5 mm O.D.) coupled to a 30 cm length of narrow-bore stainless-steel tubing (125 μm I.D., 1.5 mm O.D.). The narrow bore tubing was inserted into a 10 cm length of copper tubing (1.5 mm I.D. × 4 mm O.D.). Philips thermocoax heating wire was brazed around the outside of this copper tubing. The narrow-bore tubing protruded 1.5 cm from the heating assembly. A Philips KS 4400 temperature regulator/voltage controller (Eindhoven, Netherlands) and a Fluke type 52 digital thermometer (Watford, UK) were used in conjunction with a Farnell "L" series, 120-W (variable) power supply (Wetherby, UK) to provide power to, and control the temperature of, the thermospray. A "K" type thermocouple was laser soldered 1 cm from the tip of the thermospray necessitating the use of a PCS transmitter isolation amplifier (Philips, Eindhoven, Netherlands) in order to isolate the thermocouple from the thermocoax heating wire in the thermospray.

Amino acids, saccharides and carboxylic acids were obtained from Aldrich (Milwaukee, WI, USA). Phenolic antioxidants were provided courtesy of ICI Wilton Materials Research Centre, (Cleveland, UK). HPLC-grade methanol and acetonitrile were obtained from Rathburn (Walkerburn, UK), and water was distilled prior to analysis.

RESULTS AND DISCUSSION

Interface optimisation

In order to maintain chromatographic resolution and derive maximum sensitivity from the HPLC–FT-IR interface, the area over which solutes are deposited by the thermospray should be optimised to match that of the IR beam in the DRIFT accessory (2.25 mm diameter). The deposition area depends not only on the speed of the moving substrate, but also on the thermospray temperature and height above the moving surface. The thermospray temperature and height must be optimised for a particular mobile phase composition and flow-rate. In order to measure the efficiency of thermospray deposition, a known concentration of solute was injected (in duplicate) on to the HPLC column, deposited by the thermospray on to the substrate surface, and the peak area measured for UV detection at 275 nm. The deposited solute was then dissolved in 1 ml of

chloroform–methanol (50:50), injected on to the HPLC column, and the peak area measured for UV detection at 275 nm. The thermospray deposition efficiency was then calculated as a percentage by comparison of the UV peak area absorbance for both measurements.

Irganox 565 is a phenolic antioxidant found in various plastics, and was selected as a test solution in this study to allow comparison of IR and UV absorption measurements during the investigation of the thermospray interface. The effects of varying the thermospray height on the IR absorbance of Irganox 565 at 2915 cm^{-1} and the thermospray deposition efficiency, are shown in Figs. 3 and 4, respectively. It is clear that both parameters improve as the thermospray approaches the substrate surface. The area over which the solute is deposited on the substrate surface decreases with thermospray height and provided the thermospray temperature is sufficiently high, it is uniformly deposited. If the thermospray temperature is too low, solute may be deposited in a non-uniform manner in the form of "rings", with high concentrations of solute at the outer edges of these "rings".

The effects of varying the thermospray temperature on the magnitude of the absorbance at 2915 cm^{-1} and the thermospray deposition efficiency, are illustrated in Figs. 5 and 6, respectively, for Irganox 565. The results indicate that the greatest IR absorbance is obtained at a thermospray temperature of 158°C. The deposition efficiency remained high at lower thermospray tempeatures because a "wet" spray was produced and more solute adhered to the substrate surface. Unfortunately, a "wet" spray also caused solute spreading and hence, a reduced absorbance. The initial increase in the absorbance is mainly due to a reduction in solute "spot size" on the substrate surface. Solute spreading and "ringing"' effects are eliminated with increased thermospray temperature and an optimum spot size

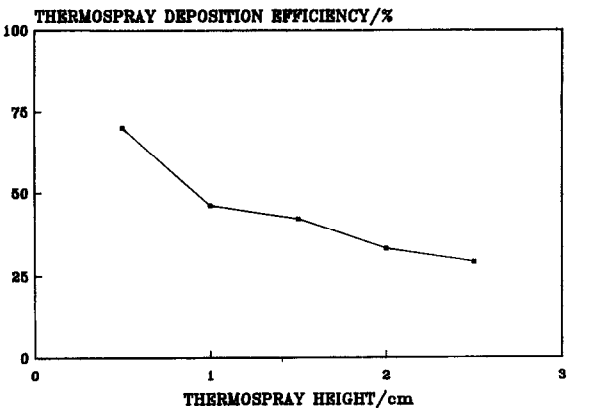

Fig. 4. Effect of varying the thermospray height on the thermospray deposition efficiency for the analysis of Irganox 565. Conditions as in Fig. 3.

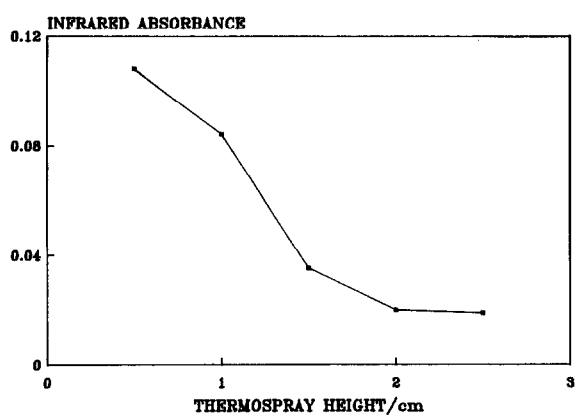

Fig. 3. Effect of varying the thermospray height on the magnitude of the IR absorbance at 2915 cm^{-1} for the analysis of Irganox 565 obtained using the MCT detector. Conditions: mobile phase, 100% methanol; flow-rate 1 ml min^{-1}; sample concentration, 300 μg ml^{-1}; column, S50DS2; thermospray temperature, 154°C (controlled to ± 0.5°C); substrate speed, 1.5 cm min^{-1}.

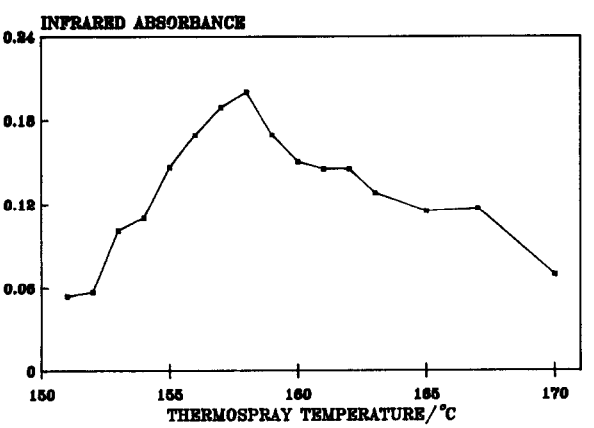

Fig. 5. Effect of varying the thermospray temperature on the magnitude of the IR absorbance at 2915 cm^{-1} for the analysis of Irganox 565. Conditions as in Fig. 3 except that the thermospray height was 0.5 cm.

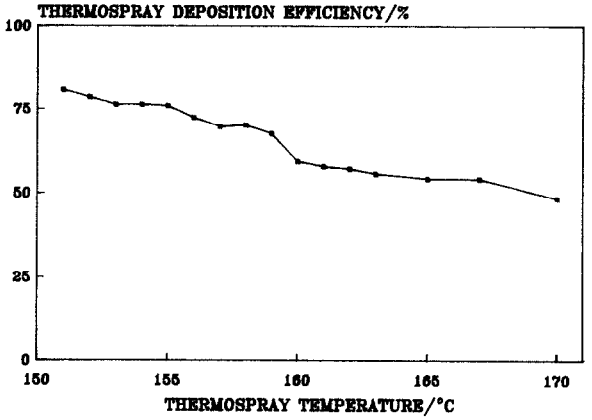

Fig. 6. Effect of varying the thermospray temperature on the thermospray deposition efficiency for the analysis of Irganox 565. Conditions as in Fig. 3 except that the thermospray height was 0.5 cm.

of 3.5 × 2.5 mm was achieved at 158°C. The optimum thermospray temperature changes if any alteration is made to the composition of the mobile phase. An increase in the thermospray temperature is accompanied by a corresponding increase in the back pressure to the HPLC pump from the thermospray. This pressure increase suggests that solute may progressively coat the inner walls of the thermospray tubing, and this may be the reason why the thermospray deposition efficiency never reaches 100%. It is also possible that some solute may be lost to the atmosphere.

In order to investigate the reproducibility of thermospray deposition, a 1 mg ml^{-1} solution of Irganox 565 was injected on to the HPLC column and deposited ten times on to the moving substrate (using thermospray temperature control). The values of the IR absorbance and the percentrage thermospray deposition efficiency measurements were 0.188 ± 0.024 and 76.6 ± 10.3% (n = 10), respectively. Although, the relative standard deviation (R.S.D.) values of 12.8% and 13.5% for the absorbance and deposition measurements are relatively high, they represent a major improvement in reproducibility of thermospray deposition compared to the performance without thermospray temperature control (typical R.S.D. levels of 50%). Further improvements to the interface design and temperature control should give additional improvement in the deposition reproducibility.

Quantitative analysis

The general trend in the literature when outlining the detection limit performance of a proposed solvent elimination interface for HPLC–FT-IR is to quote the minimum identifiable quantitity (MIQ) of a particular compound. However, if the magnitude of the chromatographic injection volume is a limiting factor and relatively low mobile phase flowrates are used [as in narrow-bore HPLC (7–10)], and no solute preconcentration techniques are applied, it is more relevant to discuss detection limits in terms of concentration of solute in the injected liquid.

Fig. 7 shows the calibration graph for the HPLC analysis of Irganox 565 at various concentrations, using FT-IR spectrometric detection. A linear relationship is indicated between the IR absorbance at 2915 cm^{-1} and the Irganox 565 concentration.

Fig. 8 shows an overlay of FT-IR spectra for Irganox 565 obtained after thermospray deposition of a 50 $\mu g\ ml^{-1}$ concentration and direct deposition of a 500 $\mu g\ ml^{-1}$ standard on to the moving substrate. A comparison of the spectra indicates that no degradation of the Irganox 565 has occurred and that identification of this solute can be achieved down to at least the 50 $\mu g\ ml^{-1}$ level. IR detection has no advantage over UV detection for this compound, particularly as the detection limit at 275 nm is around 1 $\mu g\ ml^{-1}$. The main advantage of IR detection is in the identification of solutes which exhibit less sensitive UV absorption. Quantitative

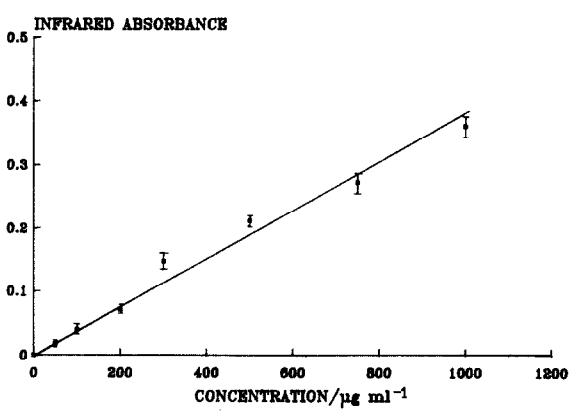

Fig. 7. Calibration graph for HPLC–FT-IR analysis of Irganox 565, indicating mean ± one S.D. of 4 results. Conditions as in Fig. 3 except that thermospray height was 0.5 cm.

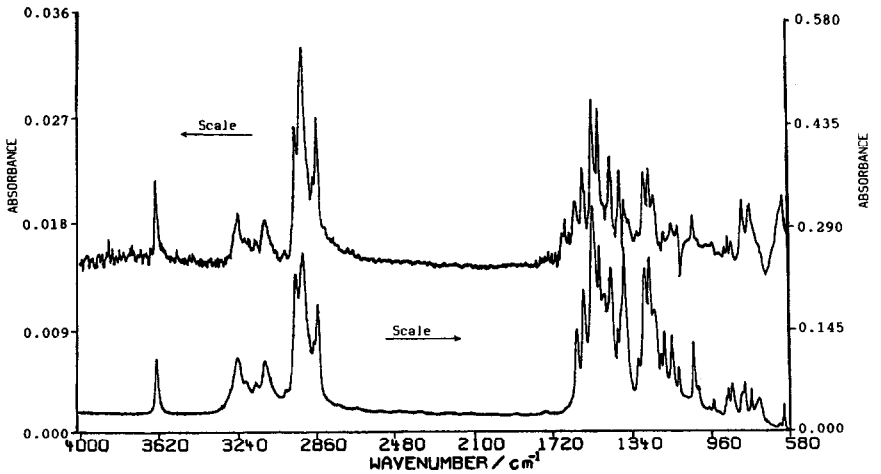

Fig. 8. Spectral overlay of the HPLC–FT-IR interface spectrum (upper) and the standard FT-IR spectrum (lower) for Irganox 565. Conditions as in Fig. 3 except that the thermospray height was 0.5 cm and the sample concentration was 50 μg ml^{-1}.

measurements were therefore obtained for a number of other compounds and it was possible to detect unambiguously individual saccharides and aliphatic carboxylic acids at 200 μg ml^{-1} and amino acids at 100 μg ml^{-1}. Lower limits of detection are likely to be achieved with better thermospray temperature control.

Qualitative analysis

The examples described so far illustrate the use of the HPLC–FT-IR interface for the analysis of compounds which can be detected by both UV-visible and FT-IR absorption spectrometry. However, some compounds do not possess a UV chromophore, and although procedures such as sample derivatization can improve detection, they are often time consuming and laborious. The refractive index (RI) detector is a common alternative "universal" detector for compounds without a UV chromophore, although it is subject to slight variations in temperature and lacks the high sensitivity of UV-visible absorption. An RI detector was used in this work to develop HPLC methods for the analysis of non-UV absorbing saccharides and aliphatic carboxylic acids. The methods were then applied to assess the potential of FT-IR detection for these compounds. Fig. 9 shows the FT-IR functional group chromatogram for the analysis of four saccharides, based on measurement of the carbonyl stretch at the wavenumber window 1750–1600 cm^{-1}. All four saccharides have been detected, however Fig. 10 indicates that exact molecular identification cannot be achieved by comparison of interface derived and standard FT-IR spectra. Although the general features of the spectra are similar, there are significant differences in the "fingerprint" region below 1500 cm^{-1}. Most notable is a loss of spectral detail for the interface spectrum.

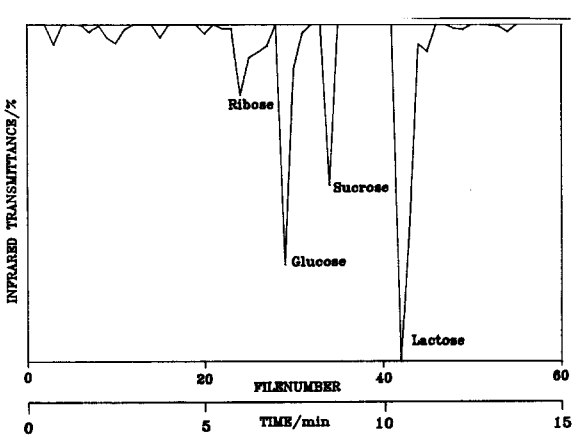

Fig. 9. FT-IR functional group chromatogram for the analysis of various saccharides obtained using the DTGS detector. Functional group, carbonyl stretch; wavenumber window, 1750–1600 cm^{-1}; conditions: mobile phase composition, acetonitrile–water (70:30); flow-rate, 1 ml min^{-1}; thermospray temperature, 180–186°C (no fine temperature control); thermospray height, 0.5 cm; column S5NH; individual saccharide concentration, 1 mg ml^{-1}; substrate speed, 1.0 cm min^{-1}.

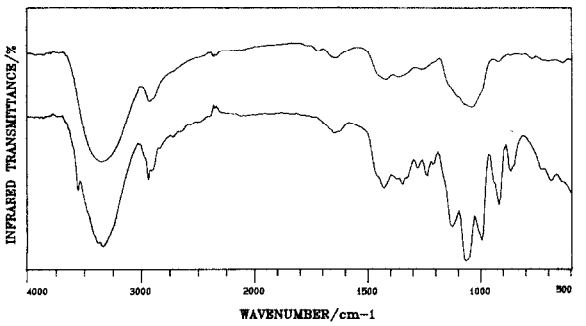

Fig. 10. Spectral overlay of the HPLC–FT-IR interface spectrum (upper) and standard FT-IR spectrum (lower) for sucrose. Conditions as in Fig. 9. Upper spectrum transmission scale multiplied by a factor of two.

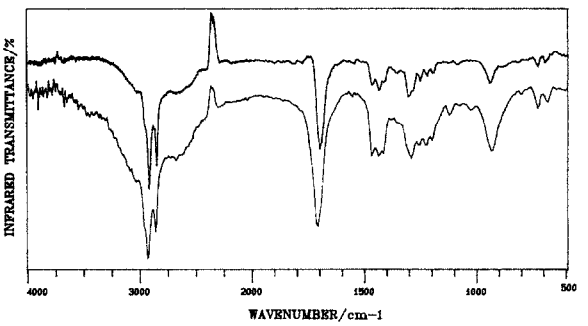

Fig. 12. Spectral overlay of HPLC–FT-IR interface spectrum (upper) and standard FT-IR spectrum (lower) for undecanoic acid. Conditions as in Fig. 11.

The differences in the spectra could be due to formation of a saccharide "glass" on the substrate surface following thermospray deposition.

The FT-IR functional group chromatogram for the analysis of five aliphatic carboxylic acids is shown in Fig. 11, based on measurement of the carbonyl stretch at 1750–1600 cm^{-1}. An overlay of the interface and standard FT-IR spectra for undecanoic acid is given in Fig. 12. No sample derivatization procedures were required for this analysis and

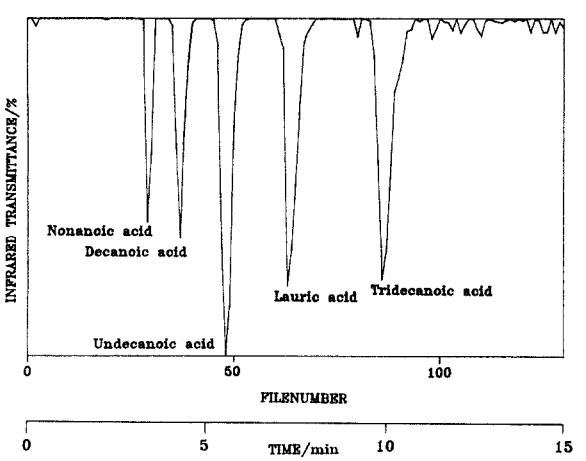

Fig. 11. FT-IR functional group chromatogram for the analysis of various aliphatic carboxylic acids obtained using the DTGS detector. Functional group, carbonyl stretch; wavenumber window, 1750–1600 cm^{-1}; conditions: mobile phase, acetonitrile–water (80:20); flow-rate, 1 ml min^{-1}; thermospray temperature, 165–170°C (no fine temperature control); thermospray height, 0.5 cm; column S50DS2; individual carboxylic acid concentration, 1 mg ml^{-1}; substrate speed, 1.5 cm min^{-1}.

molecular identification could be achieved for each of the carboxylic acids by comparison of interface and standard FT-IR spectra, indicating that passage through the thermospray caused little or no thermal sample degradation of the solutes studied. Even if sample degradation occurs for some thermally unstable solutes, this may not necessarily be a problem providing standard compounds are degraded to the same extent as the solutes in the samples and a suitably sensitive spectral feature remains. In effect, utilising this method of detection for thermally unstable solutes would be analogous to sample derivatization, without the need for preliminary sample preparation.

We have outlined previously both the "universal" and selective detection capabilities of HPLC–FT-IR [13] for the analysis of amino acids separated in a 100% aqueous mobile phase. The advantages of both detection modes has also been illustrated in the analysis of several antioxidants. Fig. 13 shows the FT-IR functional group chromatogram for the analysis of seven antioxidants. This result was obtained by monitoring the hydrocarbon stretch at 3100–2800 cm^{-1}, which is common to each solute. Only six antioxidants have been detected because of loss of chromatographic resolution derived from solute spreading during thermospray deposition. The first component to be eluted from the HPLC column after 3.3 min is in fact a composite of Irganox 3114 and Irganox 1035. The results were obtained prior to the incorporation of precise thermospray temperature control. Introduction of this feature limits solute spreading effects, and therefore

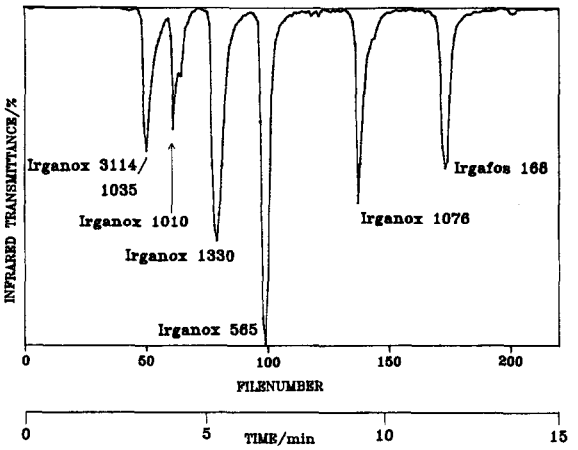

Fig. 13. FT-IR functional group chromatogram for the analysis of various phenolic antioxidants obtained using the DTGS detector. Functional group, hydrocarbon stretch; wavenumber window, 3100–2800 cm^{-1}; conditions as in Fig. 3 except that thermospray temperature was 150–154°C (no fine temperature control) and individual antioxidant concentrations were 1 mg ml^{-1}.

should minimise the loss of chromatographic resolution derived from the interface system.

In contrast, Fig. 14 illustrates the selective detection of Irgafos 168 obtained by monitoring the wavenumber window at 1100–1050 cm^{-1} covering the C-O-P vibration. Molecular identification of Irganox 1330, by comparison of interface derived and standard FT-IR spectra, is illustrated in Fig. 15. There is good comparison of the features of both spectra indicating there has been not thermal degradation of this antioxidant during passage through the thermospray.

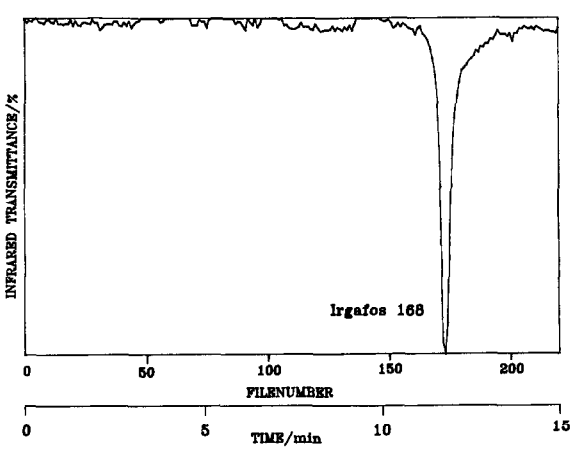

Fig. 14. FT-IR functional group chromatogram for the analysis of various phenolic antioxidants. Wavenumber window, 1100–1050 cm^{-1} (covering C-O-P vibration). Conditions as in Fig. 3 except that thermospray temperature was 150–154°C (no fine temperature control) and individual antioxidant concentrations were 1 mg ml^{-1}.

CONCLUSIONS

The development of a thermospray HPLC–FT-IR interface which can be used with most normal- and reversed-phase solvent compositions, has provided a means of obtaining qualitative information unavailable from more conventional detection methods in HPLC. The "universal" and selective detection characteristics of FT-IR detection have been illustrated for antioxidants and amino acids, and the quantitative capacilities of the system have been demonstrated. The results indicate that identification of the antioxidant Irganox 565 is possible at concentrations lower than 50 μg ml^{-1}. The interface has been applied to the analysis of non-UV absorbing species such as aliphatic carboxylic acids and saccharides, with little or no sample degradation at thermospray temperatures of 165 to 186°C. Molecular identification of each of the solutes (except saccharides) has been confirmed by comparison of interface derived and standard FT-IR spectra.

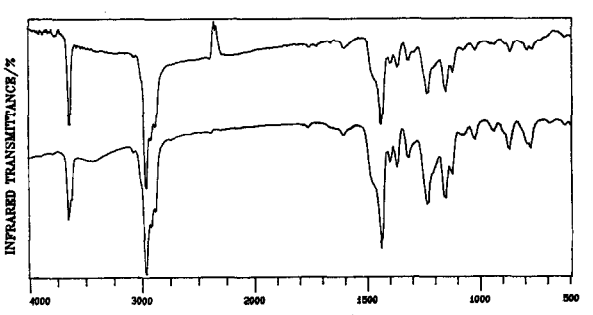

Fig. 15. Spectral overlay of HPLC–FT-IR interface spectrum (upper) and standard FT-IR spectrum (lower) for Irganox 1330. Conditions as in Fig. 3 except that thermospray temperature was 150–154°C and sample concentration was 1 mg ml^{-1}.

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